

1 ACCCGCGCGCTGCGCTGGAGACTTTCGAGAGCGCTAGCGGCCCTCGAGGCTCCCAACCACTGA
60 TGGCGCGCGGACGCGACCTTCAAGCTCGGCGGATCGCGGGGACCTCGAGGGGTGGTACT

E I

61 AGCCCAACTTCTCCCTGCGACTCGGGATCTTCAACCTCAACTGCTGGTAGTGGCTGTGC
120 TCGGGTGAAGAGGACGCTGACGCTAGAACTGGAGTTGACGACCACTACGCGAGAGC

121 GGAGTGCGGTCTGGGGGCCACCTTCCGTTTCGCAACCATGCAAGCTTCTTCCCCCTATCCC
180 CCTCAGCGCAGACCCCGGTGGAAAGCAGCGGTGGTAGCTGGAAAGGAGGGGGATAGGG

181 GCCCCAGATCTCAGGGGTGAGGGAACCCGACCTCCAAGTCCATCTTGGCCCCAG
240 CGGGTGCTAGAGTCCACATCCCTTTTGGGCTTGGAGGTTTCAGGTGTAGACCGGGTC

241 CGCGGTTGTCACAGAGTGCCTCCCTGCCCGGCTTCCCTTCTTAGGGGGCATTC
300 GCGGCCACAGGCTGTCAGCGAGGGGACGGGGCGAGAGGGAAGGAATCCCCGTAAAG

301 GTACTTGAGCAAGCACCGGGCGGACCGCATGAGGGCGCTTGGAGACTTTCGAACCAAGA
360 CATGAACTCGTCTGGCGCGGCTGGCTACTCGCGGAACCTCTGAAAGACTTTGGTCTCT

E II

361 GAGCTTCGACCTGGCTTTGCTGGAAGAGGTGAGATTGTGCAGCAGGTCGGGAACCCAGG
420 CTCGAAGCTGGACGAAAGACCTCTCTCACTTAAACGTCGTGCACGCCTTGGGTCC

421 CTGGGAGGAGGGACAGACGCTCCACTGGGGAAGACCAAGCAGGCATCTCACCGTTC
480 GACCCCTCTCCCTGTCTGCGAGGGTGACCCCTTTCTGGTCTGCTCCGTAGGAGTGGCGAAG

481 CCTCAGGTGTGAGTGAGCAGGACTTCAGACTACTGAGACAGAGCTGTCACTCACTAC
540 GGAGTCACACCTCACTGCTGTAAGGTATGACTCTGTCTTCGACAGTGGATGGATG

E III

541 CCAGCTGCACACCACTTCGGGAGGTGAGAAGCCACTGGGCTGAAGCTGTTGTGTCATCCC
600 GGTGCAAGTGTGGTAAGGCTCCACTCTTCGGGTGACCGGACTTCGGACAACAGTAGGG

601 AGGAGGCTCTTGGCCCTGCCAGCCCTTCCCTATCTCGCTGCAGCTTCCAGTCTCTCCCA
660 TTCTCGAGAACCGGGACGGTCTGGGAAGGATAGGACGAGCTGAGAGGTACAGGAGGT

661 GCCTCCTCTCCCTCTGGATGTGAGAGAGGAGAGAGGTGAACCAAGAAAGTCTATGACT
720 CGGAGGAGAGGGAGACCTACACTCTCTTCTCTTCCCACTTGGTTCTTCCAGGATACGA

721 TCAGCCCAATTCAGCTTTGTTTTCTGGCTGCCCTATACCTCCCAAGGCGCTCGCCTTG
780 AGTGGGTAAAGTCGAACCAAGAGACCGAGCGGATATGAGGAGGTTTCGGCGACGGGAC

781 GTTCTAGGGCTATGCTCCAGCAGTAGAAGAAAAAGAAAAATAGCTGATCAGAGCTGGAGAC
840 CAAGATCCCGATCAGGGTCTCATCTTTCTTTTCTTATGCACTAGTCTCGACCTCTTG

841 AAGGGAGGGGAAGGAGCTGGGTGTCTCTCCCTGTTTCTTGTGTTTAAAGCAAGGCTTG
900 TTCCCTCCCTTCTTCCGAGCCACAGAGCGGACAAAGAGCAATATTCTGCTCCGAC

Figure 1-1

Figure 1-2

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2821 -----+-----+----- 2852
TGCTTGATTCTTGTATTTCGAACCGGGT00
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Figure 1-3

Mouse Neutral Sphingomyelinase (nSMase) gene sequence

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TNGANNCTGTTAGCTCCAGNCCGGTNGGTCGCCGTNCTAGNCNNATCTNTATAGCTCTTC
1 -----+----- 60
ANCTNNGACAATCAGAGTCNGGCCANCCAGCGGCANGATCNGNNTAGANATATCGGAGAAG

GTTGCGAGCNCAATTNNNTCTCAATAAANGGATNCANCCCTATGACAGAACGTGGACCCC
61 -----+----- 120
CAACGCTCGNGTTAANNNAGAGTTATTNNCTANGTNGGATACTGTCTTGACACTGGGG

CGCCCCCANCNCANGNANACCGCGCATGGGNTGAGGTGCNCANGGTGTCTGGGGCG
121 -----+----- 180
GCGGCGGTTNGTNCNCTNTGGCGCCGTACCCNGACTCCACGNGTNCACAGACCCCGC

AGGGGTTACCTCAGCGATGGTCTTTGACACCTGAJAGCTGGAGCTTTTGAJNAGCCCCAN
181 -----+----- 240
TCCCCAATGGAGTCGCTACCAGAACTGTGGACTTTTGACCTCGAAACTTNTCGGGGTN

CACCTTCAGCTTCAGGGGCGGCTCNGGCGGCAACCGACGTGANATGCTGGGGGCTTCGA
241 -----+----- 300
GTGAAGTCTGAAGTCCCCGCCGAGNCCGCGGTGGCGTGCACNTACGACCCCCGAAGCT

CTTGGGCGGCGCAGGNTGCTGGGTGGCCATGGAJNNNACAGNACAGAGCCCGGNACACAA
301 -----+----- 360
GAAACCGGCGGTCGCNACGACCCACCGGTACTCTNNNTGTCTNTGTCTCGGGCCTGTGTT

ATANTGCGAGTCGCCANGNAAACCGCGTGGCTCTCCCCGAAACGCCNCAAGGGGCGGGA
361 -----+----- 420
TATNACGCTCAGCGGTNCNTTGGCGCACGAGGAGGGGCTTGGGGNGTTCGCCGCCCT

CCTGAGTGAGTTCTNTGGGCGGGGCTCNCATCAACTTCAAGCCTGTGTGCTGGGAAGCC
421 -----+----- 480
GGACTCACTCAAGNACCCCGCGAGNTAGTTGAAGTTCGGAACAACGACCACTTCGG

GAGCCGGGAACAAGGGAGGAACCTGTAGCCCGGCTGGGATAACCCACCGAAGGACCTA
E I 481 -----+----- 540
CTCGGCCCTTGTTCCTCTCTTGGACATCCGGGCCACGCCCTATTGGGTGGCTTCTGGAT

AGAATCTGGAACAGTCCACCCGAGATTCCTTCAGGACTCGCCGGGAGACTCTCGCATCA
541 -----+----- 600
TCTTAGACCTTGTGAGGTGGGCTCTAAGGAAGTCTCTGACGGCCGCTGAGAGCGTAAAT

GCCCGGGATTGTCAGCCGACCTCTTTCCGGGTGGAATGACGGCCCTTGTCCCACTAAG
601 -----+----- 660
CGGGCCCTAAACGTCGGCTGGAAGAAGGCCCACTTACTGCGGAACAGGGTCAATTGC

CAGSAGTCNNCCCCACCCCCAACAGCTGCGGTTCTTGGGTGGGGCAGCGCAGGATAGG
661 -----+----- 720
GTCCTCAGNNGGGGTGGGGTGTGTCGAGCGCAAGGACCCAGCCCGCTCGGTCCTATCC

GCAATAAGCCTGTGCGGCAATCCGCCTCGCCGCCCTTGCTCCGAAGCACTCCAGCCATG
721 -----+----- 780
CGTTATTTCGACACGCGCTTAGCGGAGCGGCGGAACGAGGCTTCGTGAGGTGCGGTAC

AAGCTCAACTTTTCTCTACGGCTGAGAGTTTCAATCTCAACTGCTGgtaagtaagtgct
781 -----+----- 840
TTCGAGTTGAAAAGAGATGCCGACTCTCAAAGTTAGAGTTGACGACcattcattcaaga

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Figure 2-1

E III

Figure 2-2

GGGTACTCCCTCCTTGCTCTTCTGGTTATTAAGCAAGAGTTGGTTTTAGCGGGATGAT
1741 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1800
CCCATGAGGGGAGGAACGAGAAGACCAATAATTCGTTCTCAACCAAAAGTCGCCCTACTA

E IV

1801 A G G C A G T G G C C T C T G T G T T C C C A A C A C C C A A T C A G A A A T C T T C A G C A G T G T C T A
T C C G T C A C C G G A G A C A C A A G A G G T T T G G G T A G G T C C T T T A G A A G T C G T A C A G A T 1860

1861 C A G T C T G A A T G G T T A C C C T A C A T G G T A A G G A T C T C T C C C T A T C C T T G C T A A C A C A G A C
G T C A G A C T T A C C A A T G G G A G A T G A C C A T T C C T A G A G A A G G A T A G G A A C A G T A T G T G T C T G 1920

1921 T G G A C G C A G C T T C C T G G G G C T T G G C A G G A G G G T G T C A G T A C C T G A G T T T T G T C T T C
A C C T G C G T C G A A G G A C C C G G A A C G T C C T C C C A C A T C A T G G A C T C A A A A A C A G A A G 1980

1981 T C T T G C C T G C A G T T C C A T C A T G G A G A C T G G T T C T G T G G A A A G T C T G T G G G G C T G T G G T G
A G A A C G G A C G T C A A G G T A T A C C T T G A C C A A G A C A C C C T T C A G A C C C C G A C G A C C A C 2040

EY

CTCCGTCATAAGTGACATCGTGCTCAATGCTCATCGTGACTCATGTGAGTGGGGCTAGCCAG
2041 ----- 2100
GAGGCAGATTACCTGACCAAGATTACGGATGCACTGATGACTACCCCGATCGGTC
GCTTAGGCAGTGGGTCAAGCAGCCCAATGCTATGGTGGAGAGAGAGCCCACTAGTTAGT
2101 ----- 2160
CGAATCCGTACCCAGTTCGTGGGTTACGATACCACTCTCTCTCGCGTGATCAATCA
TCTGCTGCCTGGGGATAGGCATGGGATCAGAAGCTAGCATTGGGCAAGGTTCACCCAAT
2161 ----- 2220
AGACGACGAGCCCAATTTCGTACCTAGTCTTCGATCGTAAACCGTTCGAAGTGGGTAA
CCCTGTCACTCTGCAATGTGACAGATGACAAGCTTGATTGACAGACGCTTCTCTTTGA
2221 ----- 2280
GGGACAGTGTGAGACGGTACACTGTCTACTGTGCAACTAAGTCTGTGGGAGAGAACT
TTTCACTATTTCACCTTTAGCTACATGCTGAGTACAGCCGACAGAAAGGCACTCTACTTTG
2281 ----- 2340

EVI

2341 CACA CGGTGTGGCCCAAGCTTGGGAACTGGCCCAAGTTATCATCAGTGTGTGAGCCTTGGGCT 2400
GTGTGGCACACCGGGTTCGAACCTTGACCGGGTCAAGTAGGTCACACACTCGGACCCGA
TGATGGGGCTGTGGGTGGGGACGGGGTGTAGGGATGNGNAANTTATCCTTGAAGAGGG
2401 ACTACCCCGACACCCACCCCTGCCCCAACTCCCTACNCTTNAATAGGAACCTCTCTCC
CACATAATAAGGAGAATTCTCTCTGCGCTCTTCCCCAACTAGCCACACATCCA
2461 GTGTATTATTCCTCTCTTAAAGGAGGAACGGCAGAGAAGGGGGTTCAGTGGGTGTGTAGGT 2520

E VII

AGAATGCAGATGTGGTTCTATTGTGTGGAGACCTCAATATGCACCCCAAAGACCTGGGCT
2521 +-----+-----+-----+-----+-----+-----+-----+ 2580
TCTTACGTCTACACCAAGATAACACACCTCTGGAGTTATACGTGGGGTTTCTGGACCCGA

Figure 2-3

EIX

EX

CATAGCTAAAGCTCGCTGGTGGGCTGCATTCTCTGGCTATGTGATCGTTTGGGGGCTGTC
 3301
 GTATCGATTTCGAGGGACACCCGACGTAAAGACACCGATACCTATGCAAAACCCCGACAG
 3360
 CCTCTGGTGTGCTGTGTGCTCTGGCTGCAGAGAAAGAGGCCAGGGAAGTGCCATCAT
 3361
 GGAAGACCAACAGCACACAGGACCGAGGTCCTCTTCGGGTCCCTTCACCGGTAGTA
 3420

Figure 2-4

Figure 2-5

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TCCCTTGGGGNCCNAANCCNTGGCCCGGNCCTTGGCTTTTCCCCCTTTCCCAAGNATTTTC
4261 -----+-----+-----+-----+-----+ 4320
AGGGAACCCCNNGGNTTNGGNACCGGGCCNGAACCGAAAAGGGGAAAGGGTTTCNTAAAG

AAANNTTCCCTNGGAAAANCCCTTGTNTGGNAAAACNAATNANGAACCAAGCCAAANNT
4321 -----+-----+-----+-----+-----+ 4380
TTTNNAAGGGANCTTNGGGGAACNAACCNTTTGGNTTANTNCTTGGTNCGGTTNNNA

TGCCAAANAACCNITTGGGCAAGGGGGNAAATTCANCAAGGGGNAAATTGGGAAACCC
4381 -----+-----+-----+-----+-----+ 4440
ACGGTTNTTTGGNAAACCGTTTCCCCCNTTTAAGTNGTTCNCCCNNTAAACCCCTTGGG

NTGGGTTTNCCCAAAGGGGCCNAANANT
4441 -----+-----+-----+-----+ 4468
NACCCAAAGGGTTTCCCGGGNTTNTNA

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Figure 2-6

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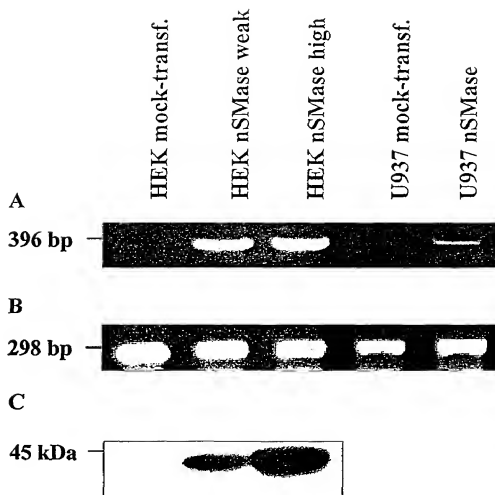


Figure 3

mnSMase "konventional" Knock Out

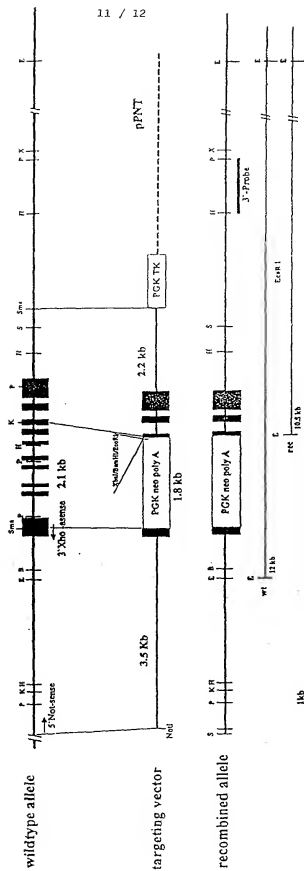


Figure 4

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Constructs for generating transgenic mouse mutants

ubiquitin promoter	nSMase	IRES	lacZ	polyA

polyA	rtTA	CMV	CMV-1	nSMase	IRES	GFP	polyA

Ubiquitin promoter: regulatory sequence of the ubiquitin gene, controlling a ubiquitous transcription.

nSMase: neutral sphingomyelinase

lacZ: lacZ, gene coding for β -galactosidase

polyA: recognition signal for the termination of transcription and polyadenylation

CMV: cytomegalovirus promoter of the cytomegalovirus gene, controlling a ubiquitous transcription.

rtTA: reverse transactivator, binds to the minimal promoter and thus controls transcription. The binding properties of the transactivator are influenced by tetracyclin. The addition of tetracyclin makes the transactivator bind to the minimal promoter and starts transcription, removal of tetracyclin prevents the binding of the transactivator to the minimal promoter and prevents transcription.

CMV-1: minimal promoter, binding of transactivator starts transcription.

IRES: *internal ribosomal entry sequence*, viral initiation signal for translation.

Figure 5